Oxidative stress and metal ions effects on the cores of phycobilisomes in *Synechocystis* sp. PCC 6803

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Abstract Inactivation of the *chlN* gene in *Synechocystis* sp. PCC 6803 resulted in no chlorophyll and photosystems when the mutant was grown in darkness, providing an in vivo system to study the structure and function of phycobilisomes (PBSs). The effects of hydrogen peroxide (H_2O_2) and metal ions on the mutant PBSs in vivo were investigated by low temperature fluorescence emission measurement. H_2O_2 induced an obvious disassembly of the cores of PBSs and interruption of energy transfer from allophycocyanin to the terminal emitter. Among many metal ions only silver induced disassembly of the cores of PBSs. Our results demonstrated for the first time that the cores of PBSs act as targets in vivo for oxidative stress or silver induced damage.

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Keywords: Energy transfer; Fluorescence emission; Metal ions; Oxidative stress; Phycobilisome; *Synechocystis*

1. Introduction

Phycobilisomes (PBSs) are the light-harvesting supramolecular complexes in cyanobacteria and several groups of eukaryotic algae [1–3]. Their function is to absorb light energy and transfer it to chlorophyll (Chl) of photosystems. PBS has two distinct regions, a core composed mainly of trimeric discs of allophycocyanin (APC) and peripheral rods composed of stacked trimeric discs. In the rods, a hexameric phycocyanin (PC) complex is always located at the rod-core linkage position, while the more distal complexes may be either PC, phycoerythrocyanin or phycoerythrin hexamers. Different linkers are specifically associated with each type of phycobiliprotein and are vital to proper assembly and functional organization of PBSs.

The PBSs can be isolated in high molarity of phosphate buffer and separated from cell debris by sedimentation [4]. Maintenance of energy transfer in isolated PBSs is dependent on several factors. It is stabilized at high phosphate buffer concentrations from 0.6 to 1.0 M, and a temperature of 20–25 °C. The energy transfer function is uncoupled at lowered phosphate buffer concentrations [5] and low temperatures [6]. However, in vivo PBSs maintain intact structure without such a concentration of phosphate or even at low temperature, suggesting higher structural and functional stability in vivo [7]. Low temperature (77 K) fluorescence measurement is a powerful tool for the study of energy transfer from PBSs to photosystems in vivo. However, it gives little insight into the energy transfer from PC to the terminal emitter in wild-type cyanobacterial cells because the emission spectra of the PBSs ensures good overlap with that of photosystems II at about 685 nm.

Synechocystis sp. PCC 6803 contains both light-dependent and light-independent Chl biosynthesis pathways [8]. Three genes (*chlL*, *chlN* and *chlB*) encode the subunits of the light-independent protochlorophyllide reductase. Mutants lacking any one of these genes are unable to synthesize Chl in the dark [9,10]. When they were propagated under light activated heterotrophic growth conditions (LAHG, in darkness except for 15 min/day of weak light), the mutant cells contained intact and functional PBSs, but essentially no Chl and photosystems [7,9,11]. Therefore, the mutant cells provide an excellent in vivo system to investigate the structural and functional characteristics of PBSs.

Active oxygen species, such as superoxide anion, hydroxyl radical, and hydrogen peroxide (H_2O_2), are generated as a result of the incomplete reduction of oxygen during respiration and photosynthesis. It is suggested that active oxygen species are extremely reactive and can cause severe damage to cell components by, for example, inactivating proteins, cleaving DNA and peroxidation of unsaturated fatty acids in cell membranes [12,13]. However, the effects of oxidative stress on the structure and function of PBSs in vivo have not been reported as far as we know. Besides, the studies of metal ions effects on PBSs in vivo are limited [14]. Also no detailed spectral analysis of the effect of metal ions on PBSs in vivo has been made.

In this work, we have studied the effects of oxidative stress and metal ions treatments on structure and function of PBSs in the *chlN* deletion mutant of *Synechocystis* sp. PCC 6803. By using mutant cells under LAHG conditions, we ruled out photosystem II fluorescence changes in low temperature fluorescence emission spectra. Our results, in this in vivo study, demonstrated H_2O_2 induced an obvious disassembly of the cores of PBSs and interruption of energy transfer from APC to the terminal emitter. In addition, among many metal ions only Ag^+ induced disassembly of the cores of PBSs.

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Abbreviations: APC, allophycocyanin; Chl, chlorophyll; H_2O_2, hydrogen peroxide; LAHG, light activated heterotrophic growth; PC, phycocyanin; PBS, phycobilisome

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2. Materials and methods

Both wild-type and mutant strains of *Synechocystis* sp. PCC 6803 were cultured at 30 °C (±1) in BG 11 medium [15]. Under LAHG conditions, the cells were kept in complete darkness except for a 15-min light period (white light at 50 μE/m^2/s) every 24 h. Aeration was provided through bubbling. The cells were harvested during the late logarithmic growth phase (around 8×10^7 cells/ml) by centrifugation at $6000 \times g$ for 7 min and resuspended in fresh medium.

Cell suspensions were mixed with either culture medium or H_2O_2 by adjusting the final concentrations of H_2O_2 from 0 to 5% (v/v). The final cell concentrations were 4×10^7 cells/ml. Re-absorption of emitted fluorescence was negligible at this cell concentration. In darkness, the samples were incubated at room temperature for 10 min. For the metal ions treatments, BG 11 medium was mixed with different concentrations of $AgNO_3$, $CaCl_2$, $CoCl_2$, $CrCl_2$, $CuSO_4$, $MnCl_2$, $NiSO_4$, or $ZnCl_2$, respectively. Cell suspensions (4×10^7 cells/ml) were incubated at the designated concentrations for 10 min in the dark.

PBSs were isolated according to Gantt [6]. Fresh cells were suspended in 0.9 M phosphate buffer at pH 7.0 containing 1 mM PMSF. An equal volume of 0.1-mm-diameter glass beads was added to the cell suspension. The cells were broken by using a Bead-Beater with three 30 s bursts at 3800 rpm. After removal of the glass beads, the homogenate was incubated in 2% (v/v) Triton X-100 for 30 min. After centrifugation ($12\,000 \times g$, 30 min), the supernatant was layered on to sucrose density step gradient (1, 0.85, 0.70, 0.55, 0.40, 0.25 M) and then centrifuged for 12 h at $120\,000 \times g$.

Chl was extracted with 100% methanol from cell pellets obtained from 1 ml of *Synechocystis* sp. PCC 6803 wild-type and mutant cell cultures ($OD_{730} = 0.8$) that had been grown in light or under LAHG conditions. The Chl content was determined according to MacKinney [16].

Absorption spectra were measured by a Pharmacia Ultra-Spec 2000 UV/Visible spectrophotometer and the relative PC amount was determined as described before [17]. Low temperature (77 K) fluorescence emission spectra were recorded in a F4500 fluorescence spectrophotometer (Hitachi, Japan). Excitation was performed at 590 nm for PC excitation. The slit widths for the excitation and emission were 10 and 3 nm, respectively.

3. Results

3.1. Spectroscopic characteristics of the *chlN*$^-$ mutant

After growth under LAHG conditions for 2 weeks, the Chl content in the *chlN*$^-$ mutant cells was about 5% of that in the wild-type. The absorption spectra of *chlN*$^-$ cells in darkness lacked the two Chl absorption peaks and remained the PC absorption peak (Fig. 1A). The mutant strain and the wild-type had nearly equal relative amounts of PBSs. Concomitant with our earlier report [9], no significant fluorescence emission that correlated with photosystems could be detected in *chlN*$^-$ mutant grown under LAHG conditions (Fig. 1B). Thus, the low Chl levels led to a disappearance of photosystems with usual fluorescence characteristics. Fig. 1C showed the 77 K fluorescence emission spectra of the wild-type and *chlN*$^-$ cells grown under LAHG conditions. Cell samples were excited at 590 nm mainly absorbed by PC. In the spectrum of wild-type cells, the peak at 650–665 nm is due to PC and APC. The peak at 685 nm is from the terminal emitter of the PBSs and the antenna CP43 of PS II. The peak at 695 nm arises from PS II while the 720 nm peak arises from PS I [18–20]. The spectrum of the *chlN*$^-$ cells only retained the fluorescence emissions (685 nm) from the terminal emitter of the PBSs, in the absence of obvious peaks (650 and 665 nm) corresponding to PC and APC. As was expected, the fluorescence emission (at 695 or 725 nm) from Chl associated with the photosystems was not found in the mutant cells. It indicated that the energy transfer to the terminal emitter of the PBSs was effective in the absence of significant amounts of Chl and photosystems.

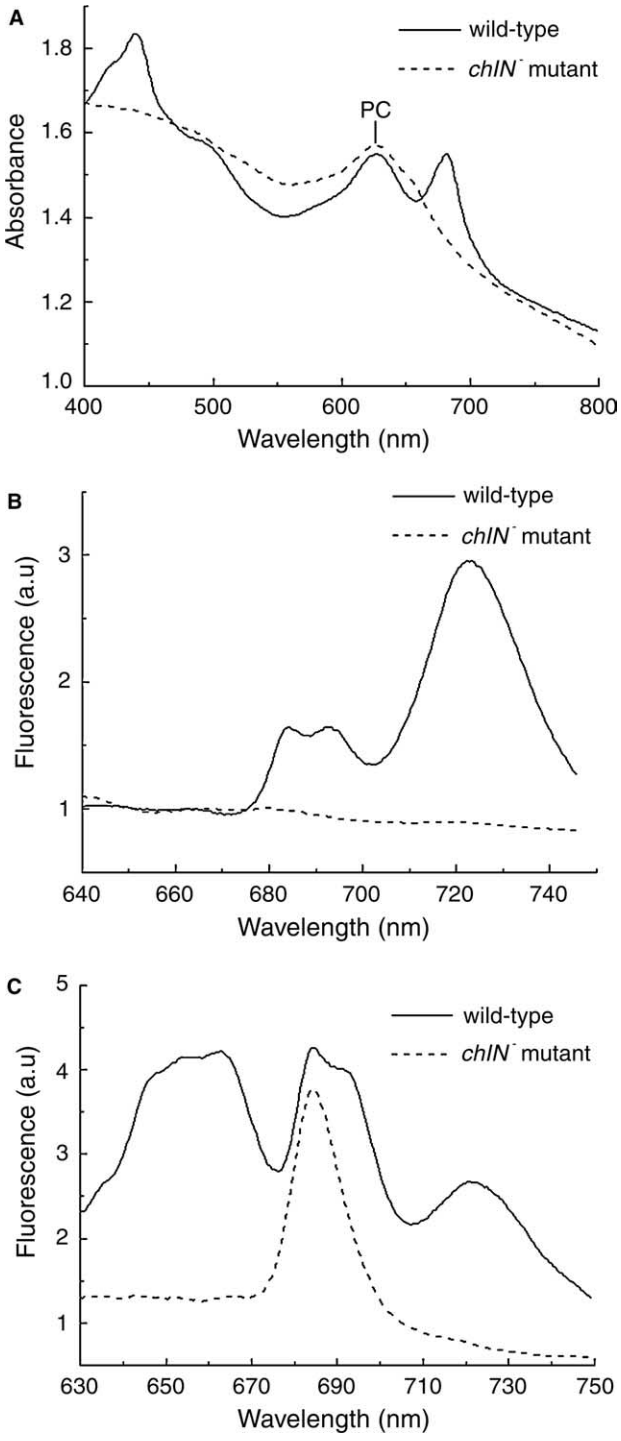

Fig. 1. (A) Whole cell absorption spectra of the wild-type and *chlN*$^-$ mutant cells grown under LAHG conditions. (B) 77 K fluorescence emission spectra of the wild-type and *chlN*$^-$ mutant cells grown under LAHG conditions. The excitation wavelength was 435 nm. The spectra were normalized at 650 nm. (C) 77 K fluorescence emission spectra of the wild-type and *chlN*$^-$ mutant cells grown under LAHG conditions. The excitation wavelength was 590 nm. The spectra were normalized at the emission maxima.

3.2. Effects of H_2O_2 on PBSs in the *chlN*$^-$ mutant

Fig. 2 showed the effects of H_2O_2 on 77 K fluorescence emission spectra of PBSs in the *chlN*$^-$ mutant. The fluorescence emission maximum of the control cells was predominantly at 685 nm. After the mutant cells were treated with 0.5% H_2O_2, a weak fluorescence emission from APC was detected around 665 nm, indicating the energy transfer from APC to the terminal emitter decreased. This APC fluorescence emission increased greatly and became the maximum emission in the cells incubated with 3% H_2O_2. It indicated that the PBSs cores were disassembled and energy transfer from APC to the terminal emitter in the core substructures was interrupted. The spectrum of cells treated with 5% H_2O_2 exhibited a major emission peaks at 659 nm and a shoulder at 680 nm, suggesting energy transfer from APC to the terminal emitter was badly damaged. However, no fluorescence emission from PC was detected at about 650 nm when the mutant cells were treated with various concentrations of H_2O_2. This suggested that the energy transfer from PC to APC was not interrupted and the rod substructures were not dissociated from the cores of PBSs. It should be noted that the fluorescence peaks at 665 and 685 nm significantly blue shifted upon the increase of H_2O_2 concentration. When the concentration of H_2O_2 increased from 0.5% to 5%, two peaks shifted to 659 and 680 nm, respectively.

The effects of H_2O_2 on room temperature emission spectra of the *chlN*$^-$ mutant cells were also determined. Although the emission bands are much broader at room temperature, these emission spectra can provide reliable information on the changes of absolute fluorescence yield from different PBS components. The addition of H_2O_2 caused increase in the APC fluorescence yield, confirming the effects of H_2O_2 on 77 K emission spectra reported above (data not shown).

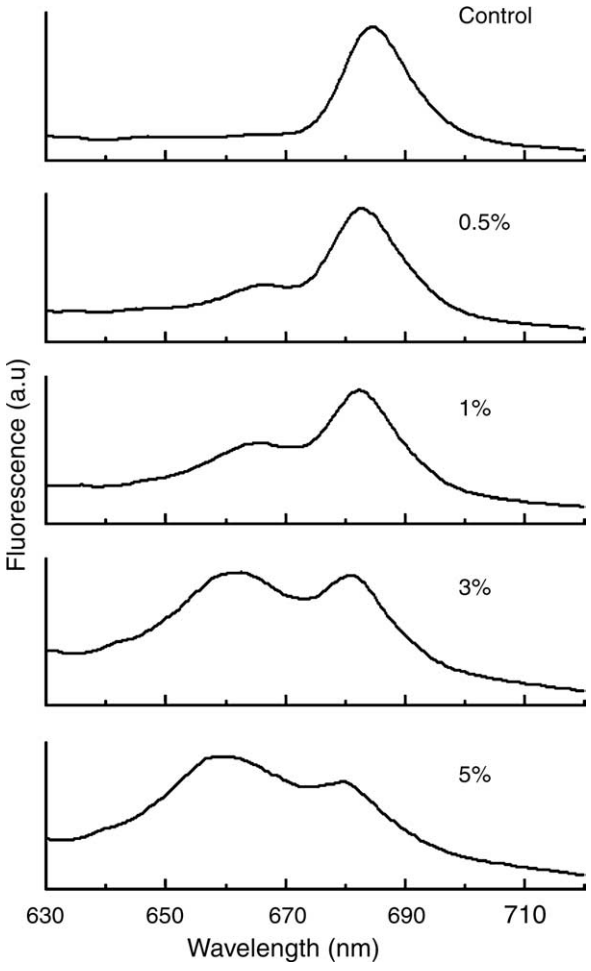

Fig. 2. Effects of H_2O_2 on 77 K fluorescence emission spectra of PBSs in the *chlN*$^-$ mutant cells grown under LAHG conditions. After treatment with various concentrations of H_2O_2 at room temperature for 10 min, the samples were frozen in liquid nitrogen. The excitation wavelength was 590 nm. The spectra were normalized at the emission maxima.

3.3. Effects of metal ions on PBSs in the *chlN*$^-$ mutant

Fig. 3 showed 77 K fluorescence emission spectra of the *chlN*$^-$ mutant treated with different metal ions. After the mutant cells were treated with 250 μM Cu^{2+}, Ca^{2+}, Co^{2+}, Cr^{2+}, Mn^{2+}, Ni^{2+}, or Zn^{2+}, the terminal emitter fluorescence peak shifted from 685 to 683 nm, but no PC and APC emission was detected to increase. The same results were observed when the concentration of metal ions increased to 1 mM. The results indicated that the structure of PBSs remained intact, since almost all energy absorbed by PC gave rise to the fluorescence characteristic of the terminal emitter. However, the spectrum of cells treated with 250 μM Ag^+ exhibited only one emission peak at about 662 nm, which originated from APC. This indicated that the cores of PBSs were disassembled and energy transfer from APC to the terminal emitter was interrupted.

Room temperature emission spectra of the mutant cells showed a loss of emission intensity by 31%, 57%, 67% and 73% after 20, 50, 80 and 100 μM Ag^+ treatments, respectively (data not shown). The results, there by, suggested that Ag^+ caused dose-dependent phycobiliproteins damage. Low temperature fluorescence emission spectra of the mutant cells treated with various concentrations of Ag^+ were measured (Fig. 4). Treatment of the cells with 20 μM Ag^+ caused a shoulder around 663 nm, the wavelength characteristic of emission from APC. Meanwhile, the peak wavelength of the terminal emitter

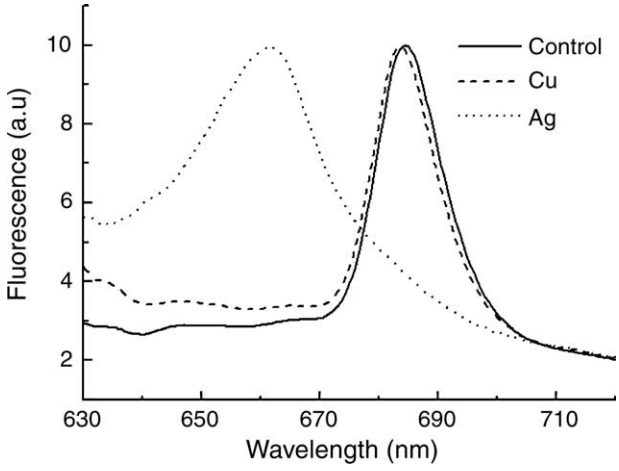

Fig. 3. Effects of metal ions on 77 K fluorescence emission spectra of PBSs in the *chlN*$^-$ mutant cells grown under LAHG conditions. After treatment with different metal ions (250 μM) at room temperature for 10 min, the samples were frozen in liquid nitrogen. Data for *chlN*$^-$ cells treated with $CaCl_2$, $CoCl_2$, $CrCl_2$, $MnCl_2$, $NiSO_4$, or $ZnCl_2$ are not presented, as they are very similar to *chlN*$^-$ cells treated with $CuSO_4$. The excitation wavelength was 590 nm. The spectra were normalized at the emission maxima.

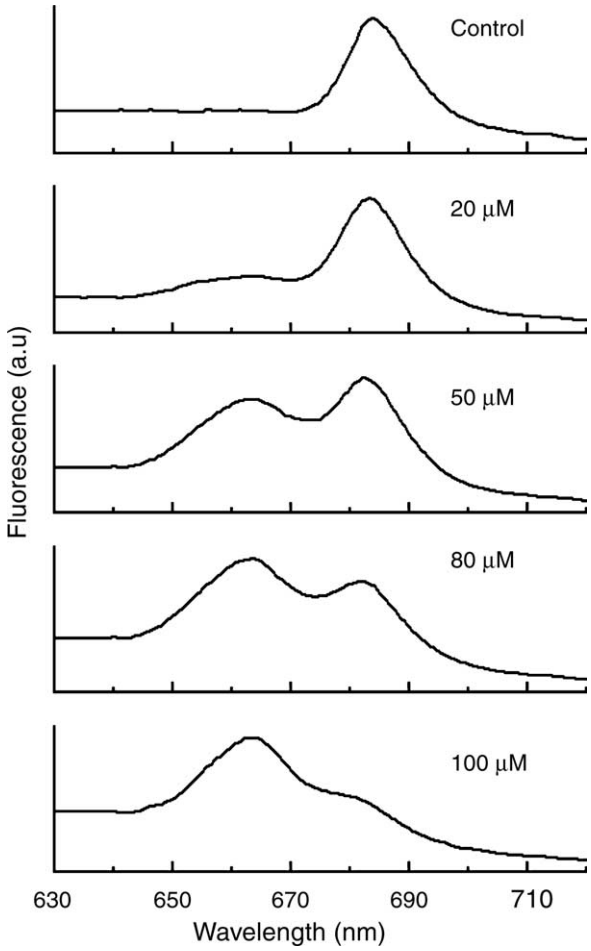

Fig. 4. Effects of Ag^+ on 77 K fluorescence emission spectra of PBSs in the *chlN*$^-$ mutant cells grown under LAHG conditions. After treatment with various concentrations of $AgNO_3$ at room temperature for 10 min, the samples were frozen in liquid nitrogen. The excitation wavelength was 590 nm. The spectra were normalized at the emission maxima.

shifted from 685 to 683 nm. Emission peak from APC became the maximum peak with 80 μM Ag^+. When the cells were incubated with 100 μM Ag^+, the 683 nm fluorescence was largely diminished, indicating that the energy transfer from APC to the terminal emitter was greatly reduced.

3.4. Characterization of PBSs isolated from H_2O_2 or Ag^+ treated cells

To further examine the H_2O_2 or Ag^+ effects on PBSs, we isolated PBSs from H_2O_2 or Ag^+ treated wild-type cells. The sucrose gradients of PBS preparations from the control and 0.5% H_2O_2 treated cells contained a single band at the position expected for complete PBSs. Interestingly, two blue bands were observed from the 1% H_2O_2 treated cells, and the nether band was at the same position as that of the control. This demonstrated that at least part of PBSs dissembled in 1% H_2O_2 treated cells. 77 K fluorescence emission spectra of these blue bands fractions were then determined (Fig. 5). The fractions from the control and 0.5% H_2O_2 treated cells produced an emission maximum at 683 nm corresponding to the terminal emitter. However, a slight emission shoulder at 657 nm was detected in the fluorescence spectrum of the PBSs from 0.5% H_2O_2 treated cells. We assign this peak to the overlap between the emission peaks from PC (645 nm) and APC (665 nm) [3]. This 657 nm peak increased greatly in the spectrum of the nether land from 1% H_2O_2 treated cells, while the spectrum of the upper land exhibited only one major emission peak at 657 nm. In addition, PBSs from 20 or 50 μM Ag^+ treated cells by sedimentation contained a single band at the same position as that of the control, and their 77 K emission spectra also showed a prevalent shoulder at 657 nm (Fig. 6). The appearance of the 657 nm peak suggests that the energy transfer efficiency to the terminal emitter has decreased in the PBSs isolated from H_2O_2 or Ag^+ treated cells, confirming our reports above.

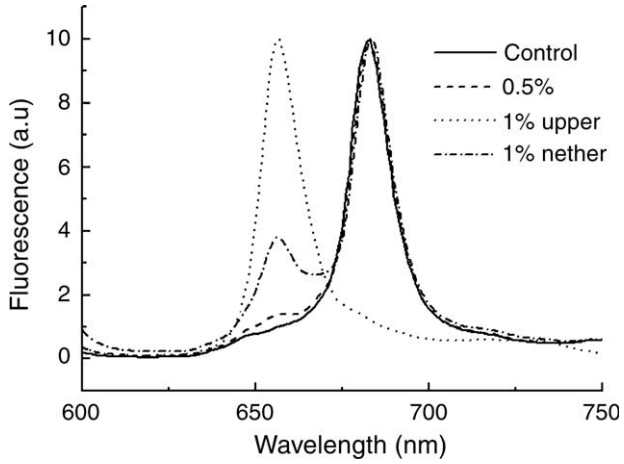

Fig. 5. 77 K fluorescence emission spectra of PBSs isolated from control and 10 min H_2O_2 treated wild-type cells. The excitation wavelength was 590 nm. The spectra were normalized at the emission maxima.

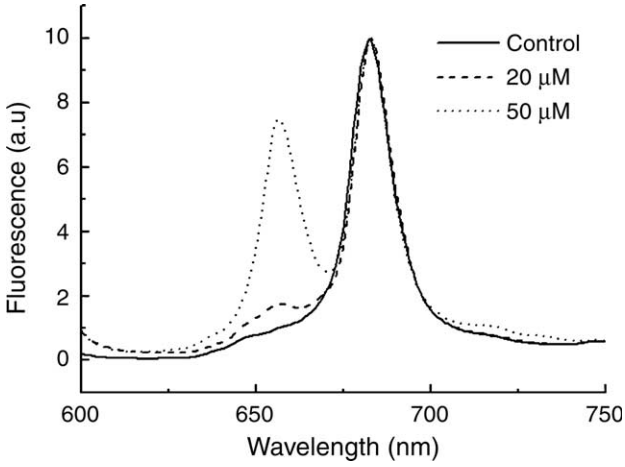

Fig. 6. 77 K fluorescence emission spectra of PBSs isolated from control and 10 min Ag^+ treated wild-type cells. The excitation wavelength was 590 nm. The spectra were normalized at the emission maxima.

4. Discussion

The two main structural features of the PBSs are the cores and the rods. The rods contain PC, phycoerythrins, and linkers. These biliproteins in the rods harvest photons, and the

excitation energy migrates through the rods and into the APC in the cores. When the rods dissociate into PC oligomers, they have similar spectra with a maximum at 650 nm in vivo [7,21,22]. No fluorescence emission from PC was found to increase in cells treated with H_2O_2 or metal ions (Figs. 2 and 3). These suggested that neither H_2O_2 nor metal ions can lead the dissociation of PC rods from the PBSs.

The APC is the main core component and has α and β subunits in a 1:1 molar ratio. There are three other core proteins with chromophores, namely, L_{CM}, β^{16}, and α^B. The L_{CM} and α^B biliproteins receive energy from APC, and then transfer it to Chl in photosystems. These two biliproteins each have a single chromophore that is red shifted compared to the other biliproteins. Their fluorescence spectra are similar to emission from intact PBSs, which allow them to transfer energy efficiently to Chl. The PBSs have tricylindrical cores. The two cylinders lie on the thylakoid membrane surface. Each cylinder is a stack of four trimer or trimer-like structures: (1) an APC trimer, $\alpha_3\beta_3$, (2) an APC trimer with a colorless linker, (3) $\alpha_2\beta_2\beta^{16}L_{CM}$, and (4) $\alpha^B\alpha_2\beta_3$ and a colorless linker. The additional cylinders, which do not lie on the thylakoid membrane, contain only APC trimers [23,24]. The fluorescence emission spectra of biliproteins are very dependent on the near surroundings of the chromophores. For example, the C-terminal domain of L_{CM} was found to induce spectral changes in APC [25]. In addition, deletion of the PB-loop in the L_{CM} subunit resulted in a blue shift at the terminal emitter emission peak, a consequence of a slight distortion of the L_{CM} around the linked phycobilin chromophore [26]. As the APC trimers have an emission maximum at 665 nm in vivo [22], the blue shift of the 665 nm peak induced by H_2O_2 or Ag^+ treatments may indicate structural changes in the APC trimers (Fig. 2). Similarly, the blue shift in the terminal emitter emission in the cells treated with either H_2O_2 or metal ions (Figs. 2 and 3) could be due to structural changes in the trimers complexes containing L_{CM} and α^B biliproteins and a consequential reduced efficiency of energy transfer from the APC to L_{CM} and α^B.

It was generally recognized that H_2O_2 has effects on protein groups as follows: oxidative modification of Met and Try, and covalent cross-linking, mainly as the formation of disulfide bonds [27–29]. Methionine sulfoxidation in oligomer was reported to resulted in an increased tendency of oligomeric dissociation, reduced exposure of hydrophobic surfaces, and altered secondary and tertiary structures [30,31]. Because the PC rods are located on the PBS surface, their peptide chains are easily exposed to H_2O_2 molecules than APC that is covered by the rods. However, fluorescence emission of APC rather than PC was induced by H_2O_2 treatments (Fig. 2). Although there is significant homology among the various biliproteins of PC and APC, more methionine residues were in APC than in PC in *Synechocystis* sp. PCC 6803. Therefore, the differential effect of H_2O_2 toward PC and APC could possibly be due to the differences in methionine residues content and protein conformation. Indeed, long time sulfur starvation could induce the alterations in the APC spectral characteristics in *Synechocystis* sp. PCC 6803, indicating the importance of the sulfur-containing amino acids in the maintenance of the structure of the PBSs cores (unpublished observations).

It was an interesting observation that among many metal ions only Ag^+ induced the fluorescence emission of APC to increase (Fig. 3). Ag^+ binds to proteins followed by reduction to free silver or formation silver sulfide with sulfur-containing amino acids [32,33]. Changes of the side chains of amino acids can have large effects on structure of APC trimers, resulting in disassembly of the cores of PBSs. Hg^{2+} induced alteration of energy transfer in PBSs by selectively affecting PC [14]. However, our results demonstrated that PC rods were not dissociated by Ag^+ treatments. We thus concluded that PBSs cores serve as a specific target of Ag^+ in vivo.

The experimental system described in this paper has opened up an opportunity to study the structure and function of PBSs under in vivo conditions. For the first time, our results demonstrated that the cores of PBSs can be targets in vivo for oxidative stress or Ag^+ induced damage. This experimental evidence might give us insights into understanding the structural changes of numerous biological macromolecules in vivo under oxidative and metal ions stress.

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References

[1] Glazer, A.N. (1989) Light guide: directional energy transfer in a photosynthetic antenna. J. Biol. Chem. 264, 1–4.
[2] Apt, K.A., Collier, J.L. and Grossman, A.R. (1995) Evolution of phycobiliproteins. J. Mol. Biol. 248, 79–96.
[3] Sidler, W.A. (1994) Phycobilisome and phycobiliprotein structures in: The Molecular Biology of Cyanobacteria (Bryant, D.A., Ed.), pp. 139–216, Kluwer Academic, Dordrecht.
[4] Yamanaka, G., Glazer, A.N. and Williams, R.C. (1978) Cyanobacterial phycobilisomes. Characterization of the phycobilisomes of *Synechococcus* sp. 6301. J. Biol. Chem. 253, 8303–8310.
[5] Gantt, E., Lipschultz, C.A. and Zilinskas, B. (1976) Further evidence for a phycobilisome model from selective dissociation, fluorescence emission, immunoprecipitation, and electron microscopy. Biochim. Biophys. Acta 430, 375–388.
[6] Gantt, E., Lipschultz, C.A., Grabowski, J. and Zimmerman, B. (1979) Phycobilisomes from blue-green and red algae. Isolation criteria and dissociation characteristics. Plant Physiol. 63, 615–620.
[7] Mao, H.B., Li, G.F., Li, D.H., Wu, Q.Y., Gong, Y.D., Zhang, X.F. and Zhao, N.M. (2003) Effects of glycerol and high temperatures on structure and function of phycobilisomes in *Synechocystis* sp. PCC 6803. FEBS Lett. 553, 68–72.
[8] Beale, S.I. (1994) Biosynthesis of cyanobacterial tetrapyrrole pigments: hemes, chlorophylls, and phycobilins in: The Molecular Biology of Cyanobacteria (Bryant, D.A., Ed.), pp. 519–558, Kluwer Academic Publishers, Dordrecht.
[9] Wu, Q.Y. and Vermaas, W. (1995) Light-dependent chlorophyll *a* biosynthesis upon *chlL* deletion in wild type and photosystem I-less strains of the cyanobacterium *Synechocystis* sp. PCC 6803. Plant Mol. Biol. 29, 933–945.
[10] Wu, Q.Y., Yu, J.J., Zhao, N.M. and Vermaas, W. (1999) Effects of chlorophyll availability on fluorescence components of photosystems in the ORF469 deletion mutant of cyanobacterium. Tsinghua Sci. Technol. 4, 1544–1550.
[11] Yu, J.J., Wu, Q.Y., Mao, H.B., Zhao, N.M. and Vermaas, W. (1999) Effects of chlorophyll availability on phycobilisomes in *Synechocystis* sp. PCC 6803. IUBMB Life 48, 625–630.
[12] Cadenas, E. (1989) Biochemistry of oxygen toxicity. Annu. Rev. Biochem. 58, 79–110.
[13] Imlay, J.A. and Linn, S. (1988) DNA damage and oxygen radical toxicity. Science 240, 1302–1309.
[14] Murthy, S.D.S. and Mohanty, P. (1991) Mercury induces alteration of energy transfer in phycobilisome by selectively affecting the pigment protein, phycocyanin, in the cyanobacterium, Spirulina platensis. Plant Cell Physiol. 32, 231–237.
[15] Rippka, R., Deruelles, J., Waterbury, J.B., Herdman, M. and Stanier, R.Y. (1979) Generic assignment, strain histories and

properties of pure cultures of cyanobacteria. J. Gen. Microbiol. 111, 1–61.
[16] MacKinney, G. (1941) Absorption of light by chlorophyll solution. J. Biol. Chem. 40, 315–322.
[17] Xu, H., Vavilin, D., Funk, C. and Vermaas, W. (2002) Multiple deletions of small cab-like proteins in the cyanobacterium *Synechocystis* sp. PCC 6803. J. Biol. Chem. 279, 27971–27979.
[18] Mullineaux, C.W. (1994) Excitation energy transfer from phycobilisomes to photosystem I in a cyanobacterial mutant lacking photosystem II. Biochim. Biophys. Acta 1184, 71–77.
[19] Bruce, D., Biggins, J., Steiner, T. and Thewalt, M. (1985) Mechanism of the light-state transition in photosynthesis. IV. Picosecond fluorescence spestroscopy of *Anacystis nidulans* and *Porphyridium cruentum* in state 1 and state 2 at 77 K. Biochim. Biophys. Acta 806, 237–246.
[20] Bruce, D. and Biggins, J. (1985) Mechanism of the light-state transition in photosynthesis. V. 77 K linear dichroism of *Anacystis nidulans* in state 1 and state 2. Biochim. Biophys. Acta 810, 295–301.
[21] Su, X., Goodman Fraenkel, P. and Bogorad, L. (1992) Excitation energy transfer from phycocyanin to chlorophyll in an *apcA*-defective mutant of *Synechocystis* sp. PCC 6803. J. Biol. Chem. 267, 22944–22950.
[22] Ajlani, G., Vernotte, C., Dimagno, L. and Haselkorn, R. (1995) Phycobilisome core mutants of *Synechocystis* PCC 6803. Biochim. Biophys. Acta 1231, 189–196.
[23] MacColl, R. (1998) Cyanobacterial phycobilisomes. J. Struct. Biol. 124, 311–334.
[24] MacColl, R. (2004) Allophycocyanin and energy transfer. Biochim. Biophys. Acta 1657, 73–81.
[25] Gottschalk, L., Lottspeich, F. and Scheer, H. (1994) Reconstitution of an allophycocyanin trimer complex containing the C-terminal 21–23 kDa domain of a core-membrane linker polypeptide L_{CM}. Z. Naturforsch. C 49, 331–336.
[26] Ajlani, G. and Vernotte, C. (1998) Deletion of the PB-loop in the L_{CM} subunit does not affect phycobilisome assembly or energy transfer functions in the cyanobacterium *Synechocystis* sp. PCC6714. Eur. J. Biochem. 257, 154–159.
[27] Levine, R.L., Mosoni, L., Berlett, B.S. and Stadman, E.R. (1996) Methionine residues as endogenous antioxidant in proteins. Proc. Natl. Acad. Sci. USA 93, 15036–15040.
[28] Levine, R.L., Moskovitz, J. and Stadtman, E.R. (2000) Oxidation of methionine in proteins: roles in antioxidant defense and cellular regulation. IUBMB Life 50, 301–307.
[29] Stadman, E.R. and Levine, R.L. (2000) Protein oxidation. Ann. NY Acad. Sci. 899, 191–208.
[30] Gustavsson, N., Harndahl, U., Emanuelsson, A., Roepstorff, P. and Sundby, C. (1999) Methionine sulfoxidation of the chloroplast small heat shock protein and conformational changes in the oligomer. Protein Sci. 8, 2506–2512.
[31] Abulimiti, A., Qiu, X., Chen, J., Liu, Y. and Chang, Z. (2003) Reversible methionine sulfoxidation of *Mycobacterium tuberculosis* small heat shock protein Hsp16.3 and its possible role in scavenging oxidants. Biochem. Biophys. Res. Commun. 305, 87–93.
[32] Rabilloud, T. (1990) Mechanisms of protein silver staining in polyacrylamide gels: a ten year synthesis. Electrophoresis 11, 785–794.
[33] Merril, C.R. (1990) Silver staining of proteins and DNA. Nature 343, 779–780.